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Notes on rust fungi in China 1. Autoecious life cycle of *Puccinia tatarinovii* on *Prenanthes*

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Abstract—During investigations of rust fungi in Jilin Province, northeast China, aecial and telial stages were observed on *Prenanthes tatarinowii* in different seasons. The telial stage was identified as *Puccinia tatarinovii* by morphological observations. Life cycle connection between aecial and telial stages was clarified by field observations and molecular data. Morphology of the spermogonial and aecial stages is described based on specimens connected to telial stage. Lectotype and epitype specimens are designated.

Key words—aecial stage, ecology, morphology, spermogonium, taxonomy

Introduction

Puccinia tatarinovii was described by Tranzschel (1939) based on four specimens on *Prenanthes tatarinowii* Maxim. (*Compositae*) collected in the Far East of Russia and northeast of China (Manchuria). Although aecial, uredinial and telial stages were reported in this description no morphological characters of the aecial stage were described, and no type specimen was designated. *Puccinia tatarinovii* is morphologically similar to *Pu. orbicula* Peck & Clinton recorded on *Prenanthes* spp., although verrucae on the surface of its teliospores are larger than those of *Pu. orbicula* (Tranzschel 1939, Ito 1950, Hiratsuka et al. 1992, Zhuang 2003). In China, telia of a rust fungus on *Pr. tatarinowii* were collected at Longtan mountain, Jilin, Jilin Province, northeast China in September 1923 and were reported as *Puccinia prenanthis-racemosae* P. Syd. & Syd. [= *Pu. orbicula*] (Miura 1928). Later, the rust specimens on *Pr. tatarinowii* were mostly treated as *Pu. tatarinovii* (Ito 1950, Tai 1979, Wei & Wang 1986, Zhuang 2003). However, they were sometimes reported as *Pu. orbicula* because they are morphologically similar and their host plants are in the same genus, *Prenanthes* (Tai 1979). Both rust species have also been reported to have an autoecious life cycle (Tranzschel 1939, Ito 1950, Cummins 1978, Zhuang 2003), but spermogonial and aecial stages of *Pu. tatarinovii* have not been clearly distinguished, and they have been confused with those of *Pu. orbicula* as these stages have not been found on the same plants at the same time. This is also largely because no morphological information has been recorded for the aecia of *Pu. tatarinovii,* and there is no record of aecial specimens in China, though many specimens of this species have been collected on *Pr. tatarinowii* and *Pr. macrophylla* Franch. from various areas of China (Wei & Wang 1986, Zhuang 2003). Azbukina (2005) described spermogonial and aecial stages on *Pr. tatarinowii*, but connection between aecial and telial stages of this species was not demonstrated.

During our investigation of rust fungi in Jilin Province, China, we found aecial (Fig. 1A) and telial stages (Fig. 2A) on *Pr. tatarinowii* at the same place in Jilin city, although they were observed at different times of the year. Therefore, we suspected that these stages were produced by the same species. We carried out morphological observations of these stages and also molecular analyses to confirm the connection between the aecial and telial stages. We report here the autoecious life cycle of *Pu. tatarinovii* and describe its morphology based on these specimens. We also designate an epitype specimen for this species.

Materials & methods

Specimens

Aecial and telial specimens on *Pr. tatarinowii* were collected in Hongyegu, Jiaohe, Jilin city, Jilin Province, China (43°42′13″N 127°04′18″E, alt. 537 m) in June and September, 2015, and used for morphological observations and molecular analysis. Specimens identified as *Pu. tatarinovii* and deposited in the Fungal Herbarium, Institute of Microbiology, Academia Sinica (HMAS), were borrowed for comparative morphology. Dry specimens used in the experiments were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU).

Morphological observations

Light microscopy (LM) was used to examine morphological characters including the size and shape of sori and spores. Spores or thin-sections of sori from specimens were mounted in a drop of lactophenol solution on glass slides for LM. Approximately 50 spores from each specimen were randomly chosen and the length, width, and wall thickness of spores were measured using Leica LAS X software attached to a Leica DM2000 microscope (Leica, Germany).

The surface features of spores were examined by scanning electron microscopy (SEM). For SEM, samples obtained from dry specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium using a Hitachi MC1000 Ion Sputter Coater and examined with a Hitachi SU8010 SEM operated at 5 kV.

DNA extraction and sequencing of rDNA-ITS regions

The total genomic DNA was extracted from about 200 spores obtained from a single sorus (aecidium or telium) on specimens. Spores were crushed between two sterilized glass slides and suspended in 30 µl extraction buffer [10mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% sodium dodecyl sulfate (SDS), 0.01% Proteinase K], and the suspensions were incubated at 37°C for 1 hour and 95°C for 10 min, followed by a 4°C soak (Suyama et al. 1996, Virtudazo et al. 2001). From the crude extract, 5–7 µl samples were used directly for each polymerase chain reaction (PCR). The rDNA-ITS region was amplified using primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes & Bruns 1993) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). The PCR amplifications were performed in 50 µl of mixture containing 5 µl of template DNA, 200 µl of each primer, 25 µl of Premix Taq™ (TaKaRa Taq™ Version 2.0 plus dye) (TaKaRa, Tokyo, Japan), and 18 µl of ddH_2O. Cycling conditions for amplification consisted of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 30 s and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were separated on 1% agarose gels that pulsed Nucleic Acid Stain (Beijing Dinggou Changsheng Biotechnology Co. Ltd.) and purified using the TaKaRa MiniBEST Agarose Gel DNA Exaction Kit Ver.4.0 (TaKaRa, Tokyo, Japan). Purified PCR products were cloned in pEASY®-T1 Cloning Vector (Transgene Biotech, Beijing, China) and then transferred into *Trans*1-T1 phage, resistant chemically competent cell according to the manufacturer's instructions. The positive clones were sequenced by Sangon Biotech Co., Shanghai, China.

Sequence similarity (%) of rDNA-ITS regions among specimens was calculated by the NCBI program (NCBI, USA).

Results & discussion

Life cycle

Sequences obtained from specimens of aecial stage with spermogonial (HMJAU8206) and telial stages (HMJAU8204) were used for analysis and these data were deposited at GenBank. However, DNA could not be extracted from spores obtained from the HMAS specimens. Chemical treatment of specimens in the herbarium may affect their DNA. Sequence similarity of rDNA-ITS regions (689 bp in length) between aecial and telial specimens was 100%. Therefore, both specimens are genetically identical and life cycle connection between aecial and telial stages on *Pr. tatarinowii* was confirmed. In the Jilin Province field observation, the uredinial stage was not found, although a few urediniospores were recognized in most specimens deposited in HMAS. We suspect that production of the uredinial stage is affected by climatic conditions.

From the results of DNA analysis and specimen observation, the autoecious macrocyclic life cycle of *Pr. tatarinowii* was clarified. Inoculations of plants with spores are best for clarifying life cycles, but collections of the plant are restricted on the mountain because of nature conservation and its transplantation to distant places is difficult. Therefore, we used DNA analysis instead of inoculations and confirmed that sequence analysis is a useful tool to clarifying a life cycle. Tranzschel (1939) and Azbukina (2005) reported aecia, but provided no information that these aecia were observed on the same host plants that produced telia. Therefore, our results provide the first evidence of the complete life cycle in this species.

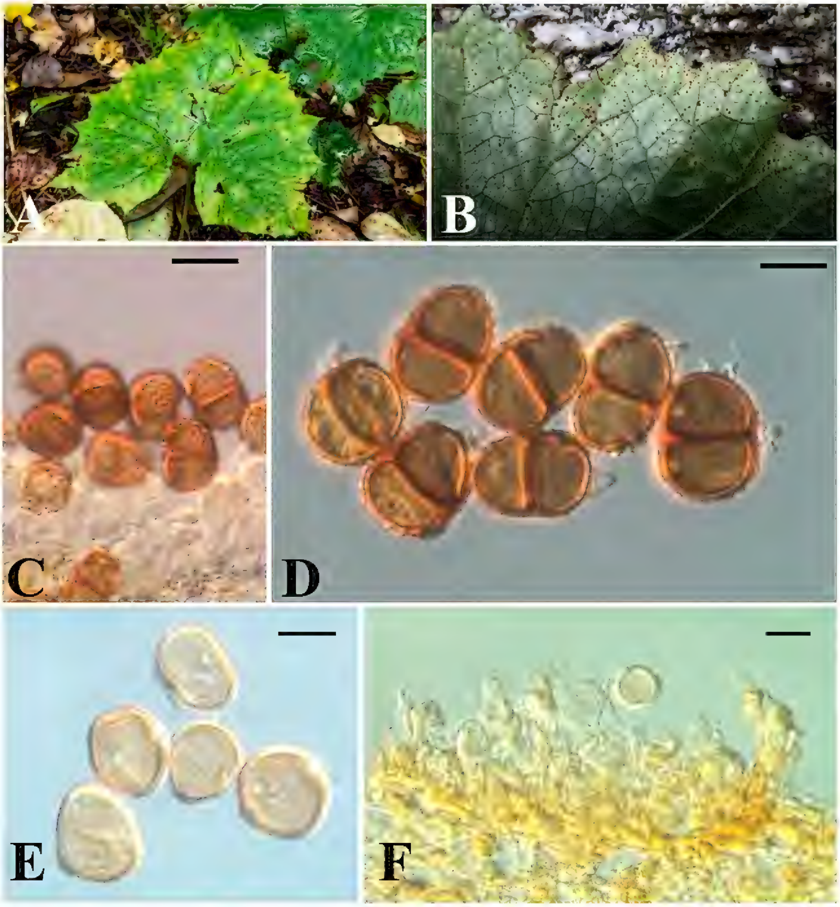

Figure 2. Uredinial and telial stages of *Puccinia tatarinovii* on *Prenanthes tatarinowii*: A, B. Telia on the upper (A) and under (B) leaf surface of *Pr. tatarinowii*; D. Vertical section of a telium; D. Teliospores; E. Urediniospores; F. Vertical section of an uredinium. Scale bars: C = 40 µm; D, F = 30 µm; E = 20 µm.

Morphology

Based on observations with specimens collected in Jilin Province (HMJAU) and specimens borrowed from HMAS, an updated description of *Pu. tatarinovii* is provided.

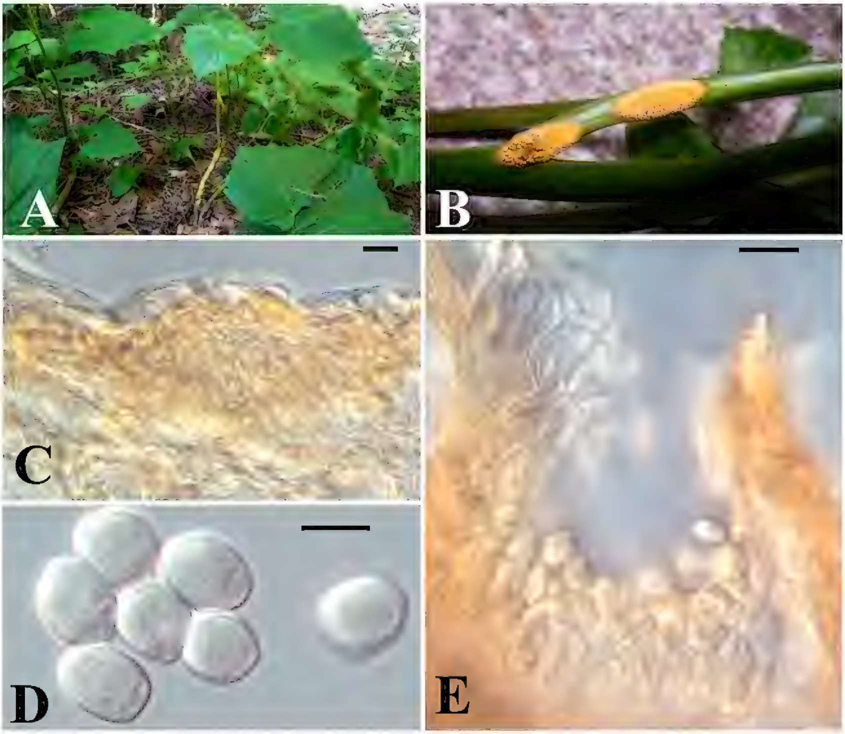

FIGURE 1. Spermogonial and aecial stages of *Puccinia tatarinovii* on *Prenanthes tatarinowii*: A, B. *Pr. tatarinowii* producing aecia in June 2015; C. Aecia produced on the stem; D. Vertical section of a spermogonium (Type 4); D. Aeciospores; E. Vertical section of an aecium. Scale bars: C, D = 20 µm; E = 30 µm.

Puccinia tatarinovii Kom. & Tranzschel, Consp. Ured. URSS: 393, 1939. FIGS 1–3

TYPE: Russia, Russian Far East, Primorsky Territory, near Vladivostok, III on *Prenanthes tatarinowii*, 10 Sep. 1913, leg. N. Djukina (**Lectotype designated here**, LE44326). China, Jilin, 0, I on *Pr. tatarinowii*, 24 June 2015, leg. M. Kakishima & J.X. Ji (**Epitype designated here**, HMJAU8206; GenBank KU955596).

Spermogonia subepidermal, Group V (type 4). Aecia mainly caulicolous, sometime hypophyllous, in small roundish groups or along veins, short, cupulate; peridia yellowish white, the margin revolute, lacerate, peridial cells polygonal or rhomboid, the outer walls striate, the inner walls verrucose, thin; aeciospores angular-globose or ellipsoid, yellowish, 13.9–22.4 × 11.4–18.0 µm (av. 17.1 × 15.2 µm), walls hyaline and verrucose, 0.3–1.6 µm (av. 0.9 µm) thick. Uredinia amphigenous, scattered, orange-yellow, globoid; urediniospores globoid or ellipsoid, 19.0–28.6 × 17.6–25.3 µm (av. 24.6 × 22.5 µm), walls uniformly thick 1.0–3.0 µm (av. 1.8 µm), echinulate. Telia mainly hypophyllous or rarely epiphyllous, especially in heavy infections, subepidermal, erumpent when mature, brown to dark brown; teliospores 2-celled, borne singly on pedicels, fusiform or oblong, sub-rotund or narrowed above, more or less constricted at the septum 25.8–44.3 × 22–32.2 µm (av. 34.1 × 23.8 µm), walls pigmented, yellowish-brown, 0.5–3.3 µm (av. 1.9 µm) thick, largely verrucose; pedicels up to 5.2 µm long.

HOSTS & DISTRIBUTION — On *Prenanthes tatarinowii*: **Russia**: Primorsky Territory; **China**: Jilin, Hubei, Beijing, Hebei, Shaanxi, Sichuan (Tranzschel 1939, Zhuang 2003, Azbukina 2005). On *Pr. macrophylla*: **China**: Sichuan (Zhuang 2003).

ADDITIONAL SPECIMENS EXAMINED —

0, I on *Pr. tatarinowii*: CHINA, SICHUAN, 17 Sep. 1989 (HMAS63916); HUBEI, 19 Aug. 1984 (HMAS50409); 27 Aug. 1984 (HMAS50414).

II on *Pr. tatarinowii*: CHINA, BEIJING, 8 June 1957 (HMAS55746); HUBEI, 4 July 1984 (HMAS57292); SHAANXI, 9 July 1988 (HMAS64179, HMAS64181).

II, III on *Pr. tatarinowii*: CHINA, HUBEI, 18 Aug. 1984 (HMAS50410); 19 Aug. 1984 (HMAS50411); 27 Aug. 1984 (HMAS50413); 29 Aug. 1984 (HMAS57289).

III on *Pr. tatarinowii*: CHINA, JILIN, 2 Oct. 2015 (HMJAU8201); 23 Sep. 2015 (HMJAU8202, HMJAU8203); 21 Sep. 2015 (HMJAU8204; GenBank KU950447); 15 Sep. 2013 (HMJAU8207); HUBEI, 27 Aug. 1984 (HMAS50412); HEBEI, 1936 (HMAS06987); Aug. 1936 (HMAS12027); BEIJING, 3 Sep. 1957 (HMAS65837); 1 Aug. 1957 (HMAS65838); 5 Oct. 1957 (HMAS25656); SICHUAN, 23 Sep. 1989 (HMAS63915, HMAS63914, HMAS63931, HMAS63932, HMAS63933); 17 Sep. 1989 (HMAS63934, HMAS63935, HMAS63936).

III on *Pr. macrophylla*: CHINA, SICHUAN, 13 Oct. 1989 (HMAS63917); 14 Oct. 1989 (HMAS63918, HMAS63919); 17 Sep. 1989 (HMAS63920, HMAS63921).

The morphological characters of uredinial and telial stages of *Pu. tatarinovii* on *Pr. tatarinowii* were similar to the descriptions of *Pu. tatarinovii* reported by Tranzschel (1939), Ito (1950), Wei & Wang (1986), Zhuang (2003), and Azbukina (2005), although there is some variation in teliospore size among these descriptions. Uredinial and telial morphologies of *Pu. tatarinovii* are quite similar to *Pu. orbicula* except for the surface structure of teliospores (Tranzschel 1939, Ito 1950, Hiratsuka et al. 1992, Zhuang 2003). Verrucae on teliospores of *Pu. tatarinovii* are larger than those of *Pu. orbicula* as reported by Tranzschel (1939) and Zhuang (2003). Therefore, verrucal size is an important character for distinguishing these species. Our observations of the aecial stage of

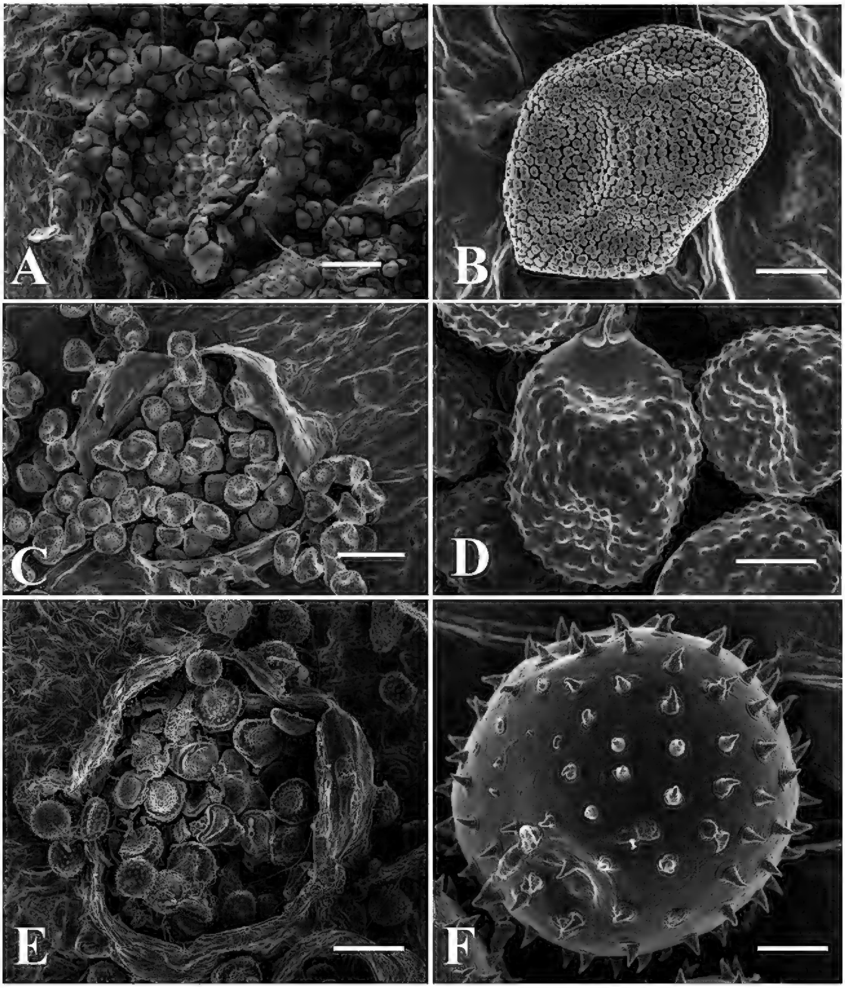

Figure 3. Aecial, uredinial, and telial stages of *Puccinia tatarinovii* on *Prenanthes tatarinowii* observed with SEM: A. Aecidium; B. Aeciospore with verrucose surface; C. Telium; D. Teliospores with large verrucae; E. Uredinium; F. Urediniospore with echinulate surface. Scale bars: A = 200 µm; B = 0.5 µm; C = 100 µm; D = 20 µm; E = 50 µm; F = 8 µm.

Pu. tatarinovii showed that its aeciospores are smaller than those of *Pu. orbicula* (23–30 × 18–22 µm) as also reported by Cummins (1978). Our observations are also similar to the description of Azbukina (2005). Therefore, size of aeciospores is an important character to recognize species. *Puccinia tatarinovii* is recorded on only two host plants, *Pr. tatarinowii* and *Pr. macrophylla*, and distributed only in China and the Russian Far East (Tranzschel 1939, Zhuang 2003, Azbukina 2005), whereas *Pu. orbicula* is widely distributed on species of *Prenanthes* (Cummins 1978, Azbukina 1984, 2005, Hiratsuka et al. 1992).

Tranzschel (1939) did not designate a type specimen of *Pu. tatarinovii* although he listed four specimens in his description. Petrak (1950: 92) also did not indicate the type for this species. Later, Azbukina (2005) selected a lectotype from these specimens but did not designate it according to the requirements of ICN Art. 7.10 (McNeill et al. 2012). Therefore, we checked specimens deposited in LE and selected as lectotype the specimen which Tranzschel separated from others as a type (personal communication from LE). Additionally, we designate a spermogonial and aecial specimen as an epitype, because aecial morphology is important to recognize the species.

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